

Urological Oncology

The use of C17,20-lyase inhibitors in prostate cancer is described by authors from Los Angeles. These agents suppress the generation of testosterone and potentially active androgenic precursors, perhaps reversing castration resistance. Abiraterone is an orally bioavailable lyase inhibitor structurally related to pregnenolone, and is currently under clinical assessment.

Another paper is presented by the same group of authors in Los Angeles addressing some of the controversies about the continual need for the traditional radical open surgical management of RCC, and evaluates the oncological principles which ensure the persistent need for this approach.

Selective blockade of androgenic steroid synthesis by novel lyase inhibitors as a therapeutic strategy for treating metastatic prostate cancer

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Accepted for publication 21 July 2005

KEYWORDS

castration refractory prostate cancer, 17 α -hydroxylase, C_{17,20}-lyase, CYP450c17, abiraterone, CB7630

THE NEED FOR NOVEL AGENTS TO TREAT PROSTATE CANCER

Prostate cancer is the most common malignancy in Western societies and the second most common cause of male cancer-related death in the UK and USA, accounting for \approx 12% of all male cancer-related deaths [1,2]. When confined to the prostate gland, the disease is curable with local therapy (radical prostatectomy, external beam radiotherapy, brachytherapy or cryotherapy). However, despite the use of PSA for screening, in 15–33% of men local therapy fails and they develop incurable metastatic disease [3,4]. Activation of the androgen receptor (AR) by androgenic steroids including testosterone and its more potent 5 α -reduced metabolite, 5 α -dihydrotestosterone (DHT), regulates the transcription of a diverse range of target genes involved in prostate cell proliferation, differentiation, and apoptosis [5]. Androgen deprivation by medical or surgical castration remains the mainstay of treatment and >90% of men with metastatic prostate

cancer initially respond rapidly and often dramatically to castration, with improvements in bone pain, regression of soft tissue metastasis, and steep declines in PSA level. However, the duration of response is frequently short (12–33 months) and in almost all patients is followed by the emergence of castration-resistant prostate cancer (CRPC) that, untreated, is invariably fatal within 9–12 months [3]. Systemic chemotherapy with docetaxel for patients with CRPC confers a modest survival advantage (2–3 months) and is effective for palliating symptoms [6], but the duration and rate of response is limited. Other chemotherapy, e.g. mitoxantrone, has not been shown to improve survival but might help with symptom control [6]. There is an urgent need for new agents that provide palliation and improve survival.

Continued androgen-dependent activation of a 'hypersensitive' AR in castrated patients secondary to AR gene amplification [7], mutations in the AR gene [5], increased AR expression [8], or alterations in AR co-repressor/co-activator function [9] appear to be important mechanisms of castration resistance. These could be reversed by suppressing circulating androgens, inhibiting the binding of biologically active androgenic steroids to the ARs or disrupting AR

generation with drugs such as HSP90 inhibitors. This review discusses the rationale behind using selective lyase inhibitors (e.g. abiraterone acetate, also known as CB7630), to suppress circulating androgens.

TARGETING THE AR

The benefit of androgen deprivation therapy (ADT) was first reported by Huggins and Hodges in 1941 [10] when surgical castration was found to give symptomatic benefit and in some cases, complete responses in metastatic prostate cancer. The role of ADT by castration has since expanded to the neoadjuvant and, more commonly, the adjuvant settings, and to patients with a high PSA level but no clinical or radiological evidence of metastatic disease [3]. Medical castration with LHRH agonists (e.g. goserelin and leuprorelin) is often a more popular alternative to orchidectomy. These act by continuous stimulation of the anterior pituitary gland, resulting in inhibition of LH secretion and suppression of testicular androgen synthesis. Antiandrogens (either steroidal, e.g. cyproterone acetate, or nonsteroidal, e.g. bicalutamide or flutamide) prevent androgen binding to the AR. These can be used in combination with LHRH analogues to inhibit binding of residual androgens to the AR to attempt 'maximum androgen blockade' [3]. However, this combined strategy has not significantly prolonged the survival of patients with advanced prostate cancer [3]. In addition, up to 30% of patients show a decrease in PSA level after stopping AR antagonists [11]. This can partly be explained by the development of AR gene mutations or increased AR expression that might cause AR antagonists to behave as weak agonists [7,8]. For many years, oestrogens, including diethylstilbestrol, were the primary medical treatment for metastatic prostate cancer, until they were superseded by LHRH agonists [3]. Now they have a role in treating castrated patients in whom castration and antiandrogen therapy have failed, but although PSA response rates in a series of phase II trials were 21–80%, the median response duration is <6 months and the concomitant use of prophylactic warfarin has not eliminated the risk of thromboembolic events [12].

The presence of AR protein expression and significant AR mRNA levels in tumour samples from patients with CRPC strongly suggest

reactivate AR signalling and AR-responsive pathways after ADT [7,13]. This is supported by preclinical models, which have suggested that, as prostate cancer cells become castration resistant (otherwise, but less appropriately known as hormone- or androgen-resistant), they acquire the ability to grow in the presence of low levels of androgens (equivalent to castrate), by the up-regulation of AR expression [8]. It was also reported that, despite castration, intraprostatic levels of testosterone and DHT in CRPC might remain sufficient to maintain tumour growth [14]. The source of these androgens is unclear, but altered regulation of enzymes involved in the synthesis and inactivation of androgens might be one cause of their accumulation. In support of this, increased expression of enzymes involved in androgen synthesis were reported in prostate cancer cells acquired from biopsies of CRPC [13,15]. These data suggest the possibility of endogenous production of steroids by CRPC. Overall, these reports indicate that the currently used ADTs fail to reverse AR signalling in CRPC and support the use of novel drugs that target the AR directly or indirectly by suppressing the generation of ligands.

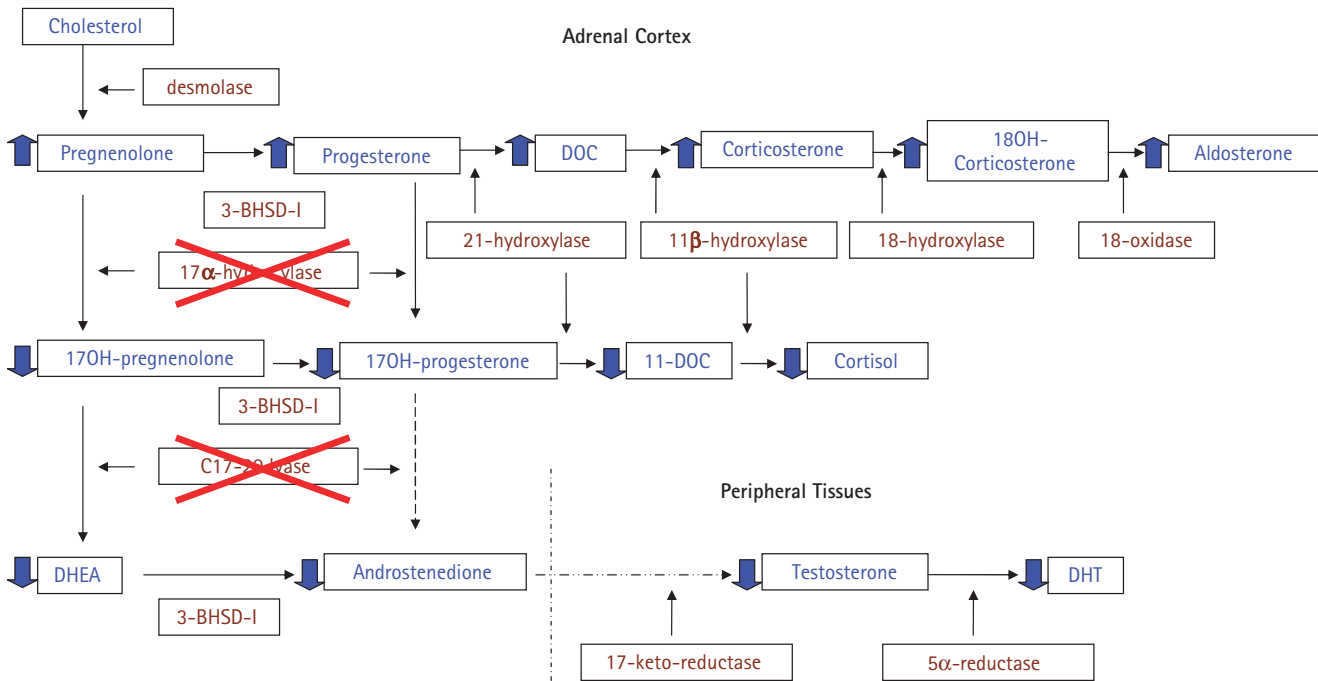
Plasma testosterone is not completely suppressed by castration, in part because of peripheral conversion of adrenal androgenic steroids to testosterone by 17-ketoreductase [3]. Adrenal androgen synthesis can be inhibited by targeting the hypothalamo-pituitary-adrenal axis or by inhibiting key enzymes involved in adrenal steroid biosynthesis. Suppression of the hypothalamo-pituitary-adrenal axis and consequently the generation of adrenal androgens by low-dose steroids has not been unequivocally shown to occur in patients with CRPC, but it could be one explanation for their anti-neoplastic activity [16,17]. However, ketoconazole, an imidazole antifungal agent that weakly and non-selectively inhibits several cytochrome P450 enzymes involved in adrenal steroid synthesis, induces a transient PSA response in 20–30% of patients with CRPC [11]. Interestingly, patients who respond to ketoconazole and subsequently progress show a significant decrease, associated with an increase with disease progression of adrenal androgens (dehydroepiandrosterone (DHEA) and androstenedione) suggesting that ketoconazole resistance is caused by adrenal androgens [11]. These clinical results lend

steroid synthesis pathway as a therapeutic strategy.

THE ROLE OF CYTOCHROME P450c17 IN ANDROGEN BIOSYNTHESIS

The two sites thought to produce most of the androgenic steroids in humans are the testis and the adrenal cortex. The principal enzymatic reaction in steroid biosynthesis involves cleavage of a six-carbon group from cholesterol by CYP450ssc (desmolase) [18]. A series of subsequent reactions catalysed by members of the cytochrome P450 family then produce the glucocorticoid and mineralocorticoid 21-carbon steroid hormones vital to human survival (Fig. 1). CYP450c17 is a single microsomal enzyme, encoded for by the single human CYP17 gene, that catalyses the two independently-regulated steroids 17 α -hydroxylase and C_{17,20}-lyase activities needed to produce the 19-carbon precursors of the sex steroids in both the adrenal cortex and the testis [18]. The 17 α -hydroxylase activity typically converts pregnenolone to 17 α -hydroxypregnenolone and progesterone to 17 α -hydroxyprogesterone, and the C_{17,20}-lyase activity converts 17 α -hydroxypregnenolone to DHEA and 17 α -hydroxyprogesterone to androstenedione (Fig. 1). The C_{17,20}-lyase activity is roughly 50 times more efficient for converting 17 α -hydroxypregnenolone to DHEA than for converting 17 α -hydroxyprogesterone to androstenedione [18,19]. The products of steroid biosynthesis, androstenedione and DHEA, are weak as androgens but in the testis can be converted to testosterone by the enzyme 17 α -hydroxysteroid dehydrogenase [20]. Castration blocks testosterone from this source but does not prevent the synthesis of adrenal androgens. These androgens might be a clinically important source of androgenic steroids for activating the AR on prostate cancer cells. Steroidogenesis in the human adrenal is divided into three morphologically and functionally distinct zones. The zona glomerulosa, located just below the adrenal capsule, does not express CYP450c17 and produces the 17-hydroxy 21-carbon steroid aldosterone, the principal mineralocorticoid, under the regulation of angiotensin II [21]. The middle layer, the zona fasciculata, expresses CYP450c17 and has abundant 17 α -hydroxylase but very little C_{17,20}-lyase activity, and produces the 17-hydroxy 21-carbon

FIG. 1. The impact of abiraterone on the adrenal steroid synthesis pathway. Abiraterone inhibits CYP450c17 17 α -hydroxylase and C_{17,20}-lyase activity (crossed out in red) and suppresses androstenedione, DHEA and their androgenic precursors (blue arrows). Suppression of cortisol and its precursors causing a compensatory rise in ACTH and excess synthesis of aldosterone and its precursors is predicted (blue arrows).



under the regulation of adrenocorticotrophic hormone (ACTH) corticotrophin [21]. The inner zona reticularis, which does not become morphologically identifiable until the onset of adrenarche, expresses CYP450c17 and has both 17 α -hydroxylase and C_{17,20}-lyase activities, and thus produces 17-hydroxy 19-carbon precursors of sex steroids [18,21]. The C_{17,20}-lyase activity of human CYP450c17 is enhanced by serine phosphorylation of CYP450c17 and by the presence of cytochrome b5. The expression of cytochrome b5 increases in the adrenal zona reticularis at the onset of adrenarche [18].

Congenital deficiencies in CYP450c17 are a rare form of congenital adrenal hyperplasia in which not only adrenal but also gonadal steroidogenesis is impaired [19]. This results in abrogation of gonadal sex steroid production and adrenal biosynthesis of cortisol and androgens. However, corticosterone synthesis is not impaired, and as corticosterone has glucocorticoid properties, patients rarely manifest symptoms of adrenal insufficiency. In fact, rodents lack CYP450c17 and use corticosterone as their principal glucocorticoid [19]. However, because

cortisol, abnormally high corticosterone production is necessary before feedback inhibition of pituitary ACTH secretion occurs, establishing a new steady state. To produce enough corticosterone to compensate for the absence of cortisol, more intermediate steroids might be generated. These include progesterone, 11-deoxycorticosterone (DOC), 18-hydroxycorticosterone and 19-nor-DOC. This ACTH-driven overproduction of mineralocorticoids often leads to hypertension, a characteristic presenting feature of this disease, usually in early adulthood [19]. At diagnosis, sexual infantilism in 46 XX females and ambiguous genitalia in 46 XY males is usually manifest and laboratory investigations will often find hypokalaemia [19]. An extremely rare disorder is isolated C_{17,20}-lyase deficiency caused by mutations that destroy most C_{17,20}-lyase activity but preserve most 17 α -hydroxylase activity. Patients do not have mineralocorticoid excess but show the consequences of absent sex steroids [19], so CYP450c17 is a logical target for the development of new drugs to treat CRPC. The most potent and selective inhibitor of CYP450c17 currently in clinical studies is

PRECLINICAL DEVELOPMENT OF ABIRATERONE ACETATE

The serendipitous discovery that some esters of 4-pyridylacetic acid are effective inhibitors of the hydroxylase-lyase enzyme in rat testis [22] led to the study of a variety of esters of 3- and 4-pyridylacetic acid and their α -alkylated derivatives on human testicular 17 α -hydroxylase/C_{17,20}-lyase [23,24]. The most potent inhibitors of human testicular 17 α -hydroxylase/C_{17,20}-lyase had, as a common structural feature, the 17-(3-pyridyl) substituent that contains nitrogen capable of forming a co-ordinate bond with the haem iron of the enzyme (Fig. 2) [23,24]. The 3-pyridyl substituent results in a several orders more potent inhibition of CYP450c17 than the 2-pyridyl and 4-pyridyl substituents [23]. The double bond in the 16,17-position of the steroidal skeleton is essential for the irreversible inhibition of CYP450c17 [25]. Two compounds, CB7598 (abiraterone), which is closely related structurally to the natural substrate pregnenolone, and CB7627, were identified as the most potent, and were selected for further development at the Institute of Cancer Research in London. With

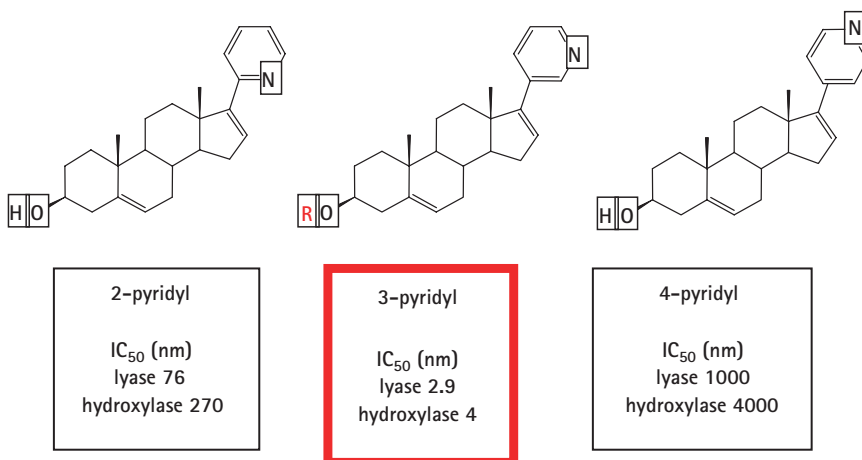
TABLE 1 Summary of phase I evaluation of abiraterone acetate

Variable	Phase I study patient population		
	Single dose in castrate men	Single dose in intact men	Continuous dosing (12 days) in intact men
Number of patients	16	4	6
Dose levels, mg	10, 30, 100, 200, 500	200, 500	500, 800
Testosterone level at inclusion, nmol/L	<2	>9	>9
Effect on testosterone (T)	Target suppression at 500 mg	Suppression with nadir on second day, recovery 6–9 days.	Suppression with nadir on first to third day and sustained for up to 9 days
Effect on androstenedione	Same as for T	Same as for T	Same as for T
Effect on LH	Suppressed at inclusion	Maximal rise on third day, recovery by 10th day	Maximal rise on third day

several times more potent inhibitors of rat hydroxylase/lyase activity than ketoconazole [23,24]. Unlike CB7627, abiraterone was a highly selective inhibitor of CYP450c17 and so was chosen as the main candidate for further development [24]. However, due to poor bioavailability and a susceptibility to hydrolysis by esterases, a prodrug for abiraterone was sought [26,27]. The amide was a several orders less potent inhibitor of hydroxylase/lyase [24] but the 3 β -O-acetate form (abiraterone acetate, CB7630) (Fig. 2) was resistant to esterases and *in vivo* was rapidly deacetylated to abiraterone resulting in potent inhibition of CYP450c17 [26,27].

When adult mice given abiraterone acetate or ketoconazole i.p. daily for 14 days were compared to a control group dosed with vehicle alone, abiraterone significantly reduced plasma testosterone concentrations for at least 24 h despite a four-fold increase in LH concentrations [27]. This was associated with reduced weights of the ventral prostate (48%) and seminal vesicles (87%), compared to the controls. The kidneys and testis were also reduced in weight by 37% and 62%, respectively [27]. There was no weight loss in the androgen-dependent organs of the group given ketoconazole [27]. There was a marked increase in the weight of the adrenal glands in the group administered ketoconazole, but no change in the control group or in the animals dosed with abiraterone, indicating no inhibition of corticosterone production by abiraterone. Animal toxicology studies found no effects on haematological, biochemical or renal function variables and only mild toxicities. Abiraterone acetate was therefore considered safe enough to be studied in

FIG. 2. The structure of abiraterone and abiraterone acetate (centre). The effect variations in the orientation of the pyridyl substituent have on the potency of 17 α -hydroxylase/C_{17,20}-lyase inhibition.



R = H: Abiraterone (CB7598); 17-(3-pyridyl)androsta-5,16-dien-3 β -ol

R = Ac: Abiraterone acetate (CB7630)

CLINICAL EVALUATION OF ABIRATERONE ACETATE

A series of three phase I studies were performed in men with histologically confirmed prostate cancer who had shown a biochemical (PSA) relapse after two lines of hormone treatment but for whom, at the time of participation, no alternative treatment for symptomatic or progressive disease was considered necessary (Table 1). The first in-human study investigated the effect of a single dose of abiraterone acetate in castrate patients (testosterone confirmed <2 nmol/L, 58 ng/mL) with prostate cancer in whom

Nine patients were treated in groups of three at doses of 10, 30 and 100 mg. At these doses, the plasma level of abiraterone was below the level of detection and there was no consistent effect on testosterone [28]. One patient was then given 200 mg and six of them 500 mg. There were detectable plasma levels of abiraterone at doses of >200 mg [28]. Abiraterone at 500 mg suppressed testosterone concentrations, to <0.14 nmol/L (4 ng/mL), or by 75% when baseline testosterone levels were >0.6 nmol/L (20 ng/mL), and androstenedione concentrations. Suppression was sustained from the second to the fifth day after abiraterone acetate.

17 α -hydroxyprogesterone. This first study was followed by a second investigating the effect of a single dose of abiraterone acetate in intact men with prostate cancer who had previously been treated with an antiandrogen and an LHRH agonist. One patient was given 200 mg, but the testosterone level was not suppressed. Three patients were then given 500 mg, and in all three there was a reduction in testosterone level of more than half from baseline. The testosterone nadir was on the second day after therapy, with recovery to pretreatment levels 6–9 days later. There was a corresponding compensatory surge in LH levels, maximal on the third day, with recovery to pretreatment levels by the 10th day. There was no change in cortisol levels in either study [28]. The third and last in this series of phase I evaluations investigated 12-day continuous dosing in intact men with prostate cancer who had previously received antiandrogens and LHRH agonists. The duration of drug treatment was limited by a lack of availability of the drug. Three patients were given 500 mg daily and another three were given 800 mg daily. The testosterone level was suppressed after the first day of dosing and was sustained for 3 days, and then reversed by a rise in LH. The patients given 800 mg appeared to show greater suppression of testosterone but as in the 500 mg cohort, the effect of abiraterone was insufficient to offset the rise in LH. All six patients had a reduced cortisol response to ACTH stimulation on the 11th day after dosing, suggesting reduced adrenocortical reserve. There was a mild reduction in evening cortisol levels also in the three patients given 800 mg but there were no clinical manifestations of adrenocortical insufficiency [28]. Pharmacokinetic studies suggested good oral bioavailability at doses of >200 mg. The mean (SD) T_{max} for abiraterone was 2.70 (2.71) h with an elimination half-life of 27.6 (20.17) h, supporting once-daily oral dosing. There was an inter-patient 10-fold variation in the area under the curve for a given dose, making analysis of dose-dependent pharmacokinetic relationships difficult. Several factors could have caused this wide level of variation. As with all oral drugs, absorption might have been altered by residual food in the stomach despite a 2-h fast, by intrinsic inter-individual differences in upper gastrointestinal pH, by concomitant medication(s) effecting gastric pH, or by variable first-pass hepatic metabolism. Abiraterone acetate was well tolerated, and

or biochemical changes were reported. Mild mood variations, flushing attacks, testicular discomfort, and headaches were described in individual patients [28].

FUTURE DEVELOPMENT OF NOVEL LYASE INHIBITORS

Several other derivatives of naturally occurring steroidal substrates, including pregnane and androstane, are potent inhibitors of 17 α -hydroxylase/C_{17,20}-lyase [29,30]. Sa40 (17-(5-pyrimidyl)androsta-5,16-diene-3 β -ol) and its 3-acetyl derivative, Sa41, are three times more potent for *in vitro* inhibition of human CYP450c17 than abiraterone and abiraterone acetate, respectively, but have a poorer pharmacokinetic profile in *in vivo* rodent experiments [29]. Clinical assessment in patients is required. L-2 (20-hydroximinio-4,16-pregnadien-3-one), L-36 (17-(3'-pyrazolyl)androsta-4,16-dien-3-one) and L-39 (17-(5'-isoxazolyl)androsta-4,16-dien-3-one) are steroidal compounds that in addition to inhibiting 17 α -hydroxylase/C_{17,20}-lyase, also inhibit 5 α -reductase with a potency similar to finasteride [30]. They also interact with wild-type AR and the mutated AR on cells from the LNCaP cell line [30]. L-36 shows an agonistic interaction whereas L-39 is antagonistic and inhibits *in vitro* LNCaP cell growth [30]. L-39 has therefore been proposed as a candidate for further development. However, the benefit of 5 α -reductase inhibitors for treating metastatic prostate cancer is unclear [3]. Although conversion of testosterone to its more active metabolite, DHT, is inhibited, testosterone consequently accumulates and can activate the AR. Also, interaction with the AR might not be desirable as changes in the AR can lead to antagonists behaving agonistically [7,8]. Other nonsteroidal compounds, including TX-977 and its two diastereoisomers, reported to be more potent *in vivo* inhibitors of testosterone synthesis, have been associated with an increase in weight of the adrenal glands in rodent models, suggesting lower specificity of lyase inhibition vs glucocorticoid synthesis [31]. Abiraterone acetate's selectivity for CYP450c17 and its failure to interact with the AR might be preferable for treating patients with CRPC. However, at present, while the available data are encouraging with respect to side-effects and endocrine effectiveness with short-term dosing, there is no evidence of clinical

abiraterone acetate administered daily and continuously to castrate men with advanced prostate cancer progressing despite hormone treatment is planned. Concomitant castration is expected to prevent a compensatory LH rise, and sustained, profound suppression of serum testosterone and androgenic precursor levels is predicted. Patients will be closely monitored for the development of glucocorticoid insufficiency or hypertension. Synthesis of corticosterone, a precursor of aldosterone and the primary glucocorticoid in rodents, will not be inhibited by abiraterone and its continued synthesis might prevent clinical manifestations of glucocorticoid insufficiency. In fact, hypertension due to increased ACTH and not glucocorticoid insufficiency is described in congenital CYP45017c deficiency [19]. The results of these studies are keenly awaited.

CONFLICT OF INTEREST

A. Beldegrun: Vice Chairman, Board of Directors and Chairman, Scientific Advisory Board, Cougar Biotechnology.

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